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THE SIGNIFICANCE OF HEMOGLOBINEMIA AND ASSOCIATED HEMOSIDERINURIA, WITH PARTICULAR REFERENCE TO VARIOUS TYPES OF HEMOLYTIC ANEMIA

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HEMOGLOBINEMIA may be defined as the existence of extra-corpuscular hemoglobin in the plasma in excess of the highest level normally found, i.e., 5 mg. per 100 milliliters. An excessive plasma hemoglobin concentration occurs as the result of intravascular hemolysis, and is therefore a prime indication of hemolytic disease. Under some conditions the concentration of free hemoglobin may become extraordinarily high. Values above 500 mg. per 100 ml. have been observed in incompatible transfusion reactions and during the paroxysms of blackwater fever. When the concentration of plasma hemoglobin exceeds the renal threshold, hemoglobin is excreted in the urine. Since the threshold lies in the neighborhood of 90 to 100 mg. per 100 ml.,[8, 11, 14, 21] it may be surmised that a rather severe hemolytic reaction must precede the appearance of hemoglobinuria. With a threshold known to be of that order, hemoglobinuria may be considered a priori evidence of severe hemoglobinemia.

Gross hemoglobinuria, as signalled by the appearance of red or black urine, is a dramatic experience. It has served as a magnet to draw the attention of many clinicians to some rare and interesting blood dyscrasias. For all their rarity, much has been learned of the diseases which exhibit this sign: the paroxysmal hemoglobinurias, favism, blackwater fever, and transfusion reactions. Hemoglobinuria also occurs in other more common conditions. It has been observed from time to time in acquired hemolytic anemia with an abnormal antibody.[2, 15, 28] Cooley observed it in one of his first cases of erythroblastic (severe Mediterranean) anemia.[3] Hemoglobinuria in sickle-cell disease has also been noted occasionally.[14, 17]

Hemoglobinemia must always, it is believed, exist as a preliminary to hemoglobinuria. It is largely through the appearance of hemoglobin in the urine that attention has been directed to the presence of hemoglobinemia. Thus, most recorded observations have been concerned with high concentrations of plasma hemoglobin. Lesser degrees of hemoglobinemia have not often been identified; nevertheless they exist. When the plasma hemoglobin level lies between 10 and 40 mg. per 100 ml. the plasma may lack a reddish tint suggest-

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ing hemoglobinemia. This occurs, not only because the concentration of hemoglobin is slight, but also because other pigments may be present. Bilirubinemia is a fairly consistent finding in any hemolytic disease, and methemalbuminemia is commonly associated with hemoglobinemia.[8, 9] The orange bilirubin and the brown methemalbumin give the plasma a tan hue which may often mask the presence of the red oxyhemoglobin.

The presence of iron-staining pigment in the urine (hemosiderinuria) has long been identified with hemolytic anemia. Marchiafava,[18] whose name is commonly associated with nocturnal hemoglobinuria, called that disease "chronic hemolytic anemia with perpetual hemosiderinuria." Rous[25] found hemosiderin in the urine of a patient with acquired hemolytic anemia and in several patients with pernicious anemia who had received many transfusions. Stats, Wasserman, and Rosenthal[28] recently stated that in hemolytic anemia other than nocturnal hemoglobinuria, hemosiderin is usually lacking from the urine or is present only in traces.

In the present studies, observations were made of the plasma hemoglobin concentration in a variety of hemolytic and other hematologic disorders. The urine of these patients was also examined for the presence of hemosiderin.

METHODS

All glassware was cleaned with potassium dichromate cleaning solution and scrupulously rinsed. Blood was obtained by venipuncture using a syringe lightly coated with mineral oil. The syringe was allowed to fill by venous pressure and without the use of suction. The needle was withdrawn and removed, and 5 ml. of blood were allowed to run gently down the side of a test tube containing 200 units of heparin (0.1 ml. of Liquaemin-Roche-Organon). Blood and heparin were mixed by gently rolling the tube between the palms of the hands. One milliliter of the blood was centrifuged for ten minutes at 1,500 r.p.m. and the plasma withdrawn. The rest of the blood was used for fragility tests. The plasma was analyzed for hemoglobin using Ham's[12] modification of Bing and Baker's method. One gram of benzidine dihydrochloride (Merck) was dissolved in 30 ml. of hot distilled water. To this were added 50 ml. of ethyl alcohol and 20 ml. of glacial acetic acid. Hydrogen peroxide was prepared as a 0.6 per cent solution in water. These solutions were always kept refrigerated and were prepared every two to three weeks. The plasma to be tested was diluted if necessary to bring the hemoglobin concentration below 20 mg. per 100 milliliter. To 0.1 ml. of plasma in a large acid-cleaned test tube were added 2 ml. of benzidine reagent and, after five minutes, 1 ml. of the hydrogen peroxide solution. A blank was prepared from the benzidine and peroxide solutions alone. Color in the unknown developed through green and blue to lavender. Ten milliliters of 20 per cent acetic acid were then added to each tube. The intensity of the color was established by the Evelyn photoelectric colorimeter. This instrument had been standardized using hemoglobin solutions of known concentration determined by the Van Slyke method of oxygen saturation[24]; 1.36 ml. of oxygen per gram of hemoglobin was the basis for the calculation. The accuracy of the method using the Evelyn instrument was well within ± 10 per cent at levels of approximately 10 mg. of hemoglobin per 100 milliliters.

Urine was tested for hemosiderin using the Prussian-blue reaction. Fifteen milliliters of urine were centrifuged. The sediment was resuspended in about 1 ml. of the supernatant. An equal volume of 5 per cent hydrochloric acid was added, plus about 0.5 ml. of a 10 per cent aqueous solution of potassium ferrocyanide. Hemosiderin appeared as deep blue particles in the sediment. If no color was visible grossly, the sediment was examined microscopically. Hemosiderin appeared as blue-stained granules within renal epithelial cells, as amorphous sediment and, occasionally, as blue-pigmented tubular casts. The intensity of hemosiderinuria was scored as follows:

1 + Microscopic
2 + Blue granules in the tip of the centrifuge tube.
3 + Blue button in the tip of the centrifuge tube.
4 + large amounts of pigment. Centrifuging unnecessary.

The diagnosis of hemolytic anemia, in addition to the information shown in Table II, was supported by the following: erythroblastic hyperplasia of the bone marrow, reticulocytosis, anemia, acholuric jaundice, increased urinary and fecal urobilinogen, and splenomegaly. In the three cases of acquired hemolytic anemia in which no antibody was detected (Cases 5, 7, and 8), the diagnosis was established by a negative family study and the demonstration of reduced survival time of transfused normal red blood cells.

The abnormal autoantibodies were studied by various methods. The titer of autoagglutinin was determined in bovine albumin suspensions according to the technique of Neber and Dameshek.[20] Coombs' test[5] using anti-human globulin serum was also employed in most cases, as was Dacie's[6] method for detecting hemolysins active at an acid pH.

RESULTS

A. *Plasma Hemoglobin.*—

1. *Control observations* (Table I) were made on over 100 normal plasmas and on the plasmas of over 100 patients without blood dyscrasias. Without a single exception the plasma hemoglobin did not exceed 4 mg. per 100 milliliters. In most instances the value lay between 2 and 3 milligrams. The values in children, in mild Mediterranean disease, in sickle-cell trait, and in myelomatosis were usually lower than those found in normal adults.

2. *Acquired hemolytic anemia* represents a heterogenous group of diseases which have in common a shortened survival time of the red blood cell due to extrinsic factors. The term "idiopathic" acquired hemolytic anemia is commonly applied to a disease of unknown etiology in which antibodies against the patient's own red blood cells (autoantibodies) may be demonstrated in the plasma, and affixed to the patient's red blood cells. This same form of hemolytic anemia is sometimes associated with leukemia or a primary or secondary neoplasm in some part of the reticuloendothelial system. Then, it is referred to as "symptomatic" hemolytic anemia. Occasional cases are found, in which an immunologic basis for the hemolytic process cannot be demonstrated. In these, chemical, parasitic, and splenic mechanisms may be present. Acquired hemolytic anemia is often chronic, but the patients frequently become critically ill and the rate of hemolysis becomes dangerously rapid. It was during and after such hemolytic crises that most of our observations were made (Table II).

When patients were examined during severe bouts of hemolysis the plasma hemoglobin was always found to be elevated. As crises subsided, the hemoglobinemia diminished and often disappeared (Cases 1, 2, and 4). Modification of the plasma hemoglobin during the course of the hemolytic anemia was a fair index of the severity of the disease in these patients. Three patients, examined one to three years after complete recovery from severe acquired hemolytic anemia, were found to have normal plasma hemoglobin values (they are not shown on the tables). In two of these patients there was still an appreciable titer of abnormal antibody against their own red cells, but abnormal hemolysis had apparently ceased. In Case 2 (Table II) it may be noted that the plasma hemoglobin became normal after nitrogen mustard therapy, yet the patient's disease persisted. Brisk hemolysis was still evident from the anemia, jaundice,

TABLE I. PLASMA HEMOGLOBULIN VALUES IN VARIOUS BLOOD DYSCRASIAS, IN NORMAL PERSONS, AND IN PATIENTS WITH NO BLOOD DYSCRASIA

DIAGNOSIS	NUMBER OF CASES	RANGE OF PLASMA HGB MG./100 C.C.
Hereditary red-cell dyscrasias		
Sickle-cell trait	8	1-2
Mediterranean trait	23	1-3
Hereditary spherocytosis	13	1-3
Hereditary elliptocytosis	1	2
Hereditary nonspherocytic hemolytic anemia	6	2-4
Hereditary stippling	1	4
Hereditary crenation	1	5
Miscellaneous blood dyscrasias with anemia		
Pernicious anemia	6	1-4
PA plus Mediterranean trait	1	1
PA plus Hereditary spherocytosis	1	3
Same during reticulocyte response to liver therapy		6-9
Same after maximal response		3
Cirrhosis of liver with macrocytosis	2	2
Idiopathic thrombocytopenia	6	3-4
Infectious mononucleosis	4	2-4
Acute (blast) leucemia	12	3-5
Chronic leucemia	7	3-5
Hodgkin's disease	8	2-4
Myelomatosis	4	1-2
Hemachromatosis	3	2-3
Control studies		
Normal persons	over 100	1-4
Patients with no blood dyscrasia	over 100	1-4

It is probable that in all acute leucemias and many late cases of Hodgkin's disease there develops a resistance to transfusion which is due in large part to hemolysis, although clinical evidence of hemolytic disease may be lacking. Such cases are included in this table.

The values above are, for the most part, based upon repeated examinations of the patients enumerated.

and reticulocytosis. One month later his spleen and an unsuspected intestinal tumor (lymphosarcoma of the ileum) were removed. Only then did the anemia and the hemolytic syndrome disappear. Patient 2 showed a lower level of plasma hemoglobin after nitrogen mustard therapy* and a still lower level after subsequent splenectomy. Both changes were attended by some measure of clinical improvement, but the patient died suddenly during an intercurrent respiratory infection several months after our last observation. The plasma hemoglobin concentration in Case 4 fell as the red cell count was brought by transfusions from 1 million to 3.5 million. After nitrogen mustard therapy this patient had a complete remission of the hemolytic disease with complete disappearance of abnormal antibody. The remission has now persisted for about three years. In several patients with chronic acquired hemolytic anemia (Cases 6, 11, 12), ACTH (adrenocorticotropic hormone) was found to produce a well-defined remission which was either partial or complete. In each case, the abnormal plasma hemoglobin regressed during the course of therapy and disappeared earlier than any of the other signs indicating hemolytic disease. When acquired hemolytic anemia was manifested by outspoken spherocytosis

*Nitrogen mustard was used in an attempt to injure the reticuloendothelial system and so inhibit antibody formation.

(Cases 1, 3, 4, and 6) the plasma hemoglobin was generally higher and more consistently elevated than it was where no such evidence of red cell injury existed.

The cases of acquired hemolytic anemia showed no variation in the degree of hemoglobinemia before and after sleep. The plasma of one patient (Case 4) was examined just before and immediately after a severe febrile reaction to the transfusion of compatible whole blood (plasma transfusion reaction). The hemoglobin concentration before transfusion was 17.5 mg. per 100 ml.; after transfusion it was 19 milligrams.

3. *Hereditary Spherocytosis:* Thirteen patients with hereditary spherocytosis (congenital hemolytic jaundice) were studied. Nine of them were examined repeatedly. Eight were examined before and after splenectomy. One of the patients who was not subjected to splenectomy was a 65-year-old man whose hereditary spherocytosis was complicated by pernicious anemia (Table I). Excepting for this case, the plasma hemoglobin in hereditary spherocytosis was always found to be low, and usually below 3 mg. per 100 milliliters. Three of the patients, all children, were observed during the crises which not uncommonly complicate this disease.[7, 22] Two of these patients were brothers and were ill at the same time. In each of the three children, the plasma hemoglobin was determined repeatedly, and in no instance was it found to be elevated. It was, in fact, so strikingly low that the benzidine reaction turned a barely perceptible color, and values of about 1 mg. were recorded. It should be noted that the crises in these cases were associated with pancytopenia and indications of maturation arrest in marrow (aregenerative reaction).[7] No cases of hereditary spherocytosis in crisis associated with an abnormal autoantibody were observed in this series. Such patients have been studied[7, 27] and, although the plasma hemoglobin levels were not determined, it seems probable that they would have been elevated. The presence of an abnormal antibody in hereditary spherocytosis suggests that an acquired hemolytic disease has been superimposed upon the hereditary. In the few cases in which hereditary spherocytosis has been reported with hemoglobinuria,[1, 15, 23] the crisis may well have been due to the presence of abnormal antibodies. In fact, Ludke[15] identified an abnormal hemolysin in his patient's serum and Paschkis' patient had a positive Donath-Landsteiner test for cold hemolysins.[23] In both instances, the diagnosis of hereditary spherocytosis was proved in the patient after his recovery from the crisis, and also by a positive family study.

Hemosiderinuria was not observed in any of our patients with hereditary spherocytosis. It has, however, been reported to occur during hemolytic crisis in this disease.[26]

4. *Severe Mediterranean Anemia and Sickle-Cell Anemia:* All patients in this group of disease showed hemoglobinemia consistently.* The patients shown

*It was suspected that the elevated plasma hemoglobin in severe Mediterranean anemia might have been an artefact. There exists in the blood of these people tiny fragments of red cells called schistocytes.[4] These globules are not as easily sedimented by centrifuging as are red cells. The following test showed that their presence is not sufficient to produce any great difference in the values ob ained for plasma hemoglobin. Blood from patient 18 was centrifuged for five minutes at 500 r.p.m. which precipitated the red cells but not the schistocytes. This plasma was divided, and half was centrifuged again for fifteen minutes at 10,000 r.p.m. The recentrifuged specimen showed a hemoglobin concentration of 23 mg. per 100 ml., as against 25 mg. for the original plasma.

TABLE II. PLASMA HEMOGLOBIN VALUES AND URINARY HEMOSIDERIN IN VARIOUS KINDS OF HEMOLYTIC DISEASE

CASE	SEX AND AGE	DIAGNOSIS	ALBUMIN ANTIBODY	RED-CELL MORPHOLOGY	PLASMA HGB. MG./ 100 C.C.	URINARY HEMOSIDERIN
1	F56	Acquired hemolytic anemia	1:16	Moderate spherocytosis	24	++
		After splenectomy, 8 months			3	0
2	M35	Acquired hemolytic anemia	1:8	Slight spherocytosis	22	+
		Lymphosarcoma of the ileum				
		After nitrogen mustard			2	0
		After splenectomy and resection of intestinal tumor (during 1 year)		No spherocytosis	2-4	0
3	F56	Acquired hemolytic anemia	1:250 acid hemolysins also present	Marked spherocytosis; 5% erythroblasts	166	++++
		After nitrogen mustard			35	+++
		After splenectomy, 1 month			25	+++
		After splenectomy, 3 months			8	+++
4	F66	Acquired hemolytic anemia	1:64	Moderate spherocytosis	17-19	+
		After transfusions			10	+
		After nitrogen mustard (during 1 year)			2-4	0
5	F45	Acquired hemolytic anemia	0	Macrocytosis	14	+
		After splenectomy			16	+
6	M55	Acquired hemolytic anemia	1:128	Marked spherocytosis	52	+++
		After splenectomy			7	+++
		After splenectomy, 1 month			15	+++
		After splenectomy, 3 months			21	+++
		After splenectomy, 4 months			50	+++
		After splenectomy, 8 months			62	++++
		During ACTH therapy			13	+++
7	F9	Acquired hemolytic anemia	0	Poikilocytosis	7-20	+
		After splenectomy			9	+
		After splenectomy, 1 month			45	+
8	M5	Acquired hemolytic anemia	0	Slight spherocytosis	3	0
9	F23	Acquired hemolytic anemia with pregnancy (subsiding)	0 (Coombs' test +)	Essentially normal	6	+
10	F67	Acquired hemolytic anemia (Chronic. Crisis subsiding)	0 (Coombs' test +)	Essentially normal	10	++
11	F40	Acquired hemolytic anemia	1:30,000, cold; 1:64, warm	Moderate spherocytosis	34	++
		Giant follicular lymphoma				
		During ACTH therapy			3	++
12	M65	Acquired hemolytic anemia	1:32	Slight spherocytosis	20	+
		Lymphosarcoma				
		During ACTH therapy			3	0
13	F36	Acquired hemolytic anemia	0	Erythroblasts	4	0
		Chronic di Guglielmo's syndrome				
14	F61	Acquired hemolytic anemia				
		On cold days	1:8000 cold	Minimal spherocytosis	15-40	+++
		On warm days			3	+++
15	F0	Hemolytic disease of newborn	1:4	Erythroblasts	9	0
16	F34	Hemolytic disease, cause unknown without anemia	0	50% R.B.C. with Heinz bodies	32-41	++
17	F18	Acute disseminated lupus erythematosis	0	Normal	9	0

TABLE II.—CONT'D

CASE	SEX AND AGE	DIAGNOSIS	ALBUMIN ANTIBODY	RED-CELL MORPHOLOGY	PLASMA HGB. MG./ 100 C.C.	URINARY HEMOSIDERIN
18	M18	Severe Mediterranean anemia After splenectomy	0	Target cells; erythroblastosis	12-60	+
19	M13	Severe Mediterranean anemia Before and after splenectomy	0	Target cells; erythroblastosis	32	+
20	F3	Severe Mediterranean anemia After splenectomy	0	Target cells; erythroblastosis	27	+
21	M27	Moderately severe Mediterranean anemia				
		Before splenectomy	0	Target cells only	3	0
		After splenectomy	0	Erythroblastosis and target cells	25	+
22	M6	Sickle-cell anemia	0	Sickling	16	+
23	M6	Sickle-cell anemia	0	Sickling	21	+

in Table II were examined repeatedly over a period of eighteen months. The level of the plasma hemoglobin was remarkably constant. Most determinations fell between 20 and 25 mg. per 100 ml., although the range was considerably wider than this. Patient 18, for instance, on one occasion was found to have a plasma hemoglobin level of 60 milligrams. He was profoundly anemic at this time. One week later, after transfusions had improved his condition and his red cell count, the plasma hemoglobin concentration was 24 mg. per 100 milliliters.

Of particular interest was the result of splenectomy in patient 21. This young man with moderately severe Mediterranean anemia had for many years required only an occasional transfusion. Later, transfusions were required frequently and the spleen gradually became massively enlarged. Plasma hemoglobin was repeatedly determined, and was always below 3 mg. per 100 milliliters. Splenectomy was done. Following the operation, the patient experienced a remarkable clinical improvement and required no further transfusions. However, the peripheral blood which before splenectomy showed only hypochromic target cells now presented the classical erythroblastic picture of severe Mediterranean anemia. Furthermore, the plasma hemoglobin became and remained elevated. This suggested that red cells which had previously been destroyed in the spleen without increasing the plasma hemoglobin were now surviving to be hemolyzed intravascularly with consequent hemoglobinemia.

B. *Hemosiderinuria.*—

In every case showing hemoglobinemia, hemosiderinuria was always found. In general, the amount of iron-staining pigment in the urine varied with the concentration of the plasma hemoglobin. Thus, a large amount of hemosiderin was usually found in the urine of those patients whose plasma hemoglobin remained in the vicinity of 50 mg. per 100 milliliters. This was not invariable. Occasionally only a trace of hemosiderin was present. On the other hand, it was noted that with a temporary lowering of the plasma hemoglobin, i.e., during warm weather for a patient with cold hemoglobinuria or during the remission

post-splenectomy in patient 3 (Table II), the heavy discharge of hemosiderin persisted. In patients whose plasma hemoglobin was usually found to be around 25 mg. per 100 ml. (sickle-cell and Mediterranean anemia) the hemosiderin was, in most cases, less than that found with heavier hemoglobinemia. Young children with this level of plasma hemoglobin usually had only a trace of hemosiderin in their urine.

We have not found hemosiderin in the urine of patients without hemoglobinemia. An exception to this was a man with severe hemochromatosis, whom we saw at the British Postgraduate Medical School in London: hemosiderin was found in his urine. In three of our own patients with this disease there was no hemosiderinuria. Hemosiderin was absent from the urine of patients with hemolytic anemia in whom there was no hemoglobinemia (Table II).

DISCUSSION

Slight though definite hemoglobinemia, of an intensity not great enough to cause hemoglobinuria, has been demonstrated in several hemolytic diseases. Hemosiderinuria was found to be invariably associated with hemoglobinemia. The definition of the hemolytic syndrome, as it occurs in acquired hemolytic anemia, sickle-cell anemia, and severe Mediterranean anemia, might well be amplified to include a low-grade hemoglobinemia and hemosiderinuria as common characteristics.

Diagnostic Significance.—The demonstration of hemoglobinemia in these diseases is of some diagnostic value. Hemoglobinemia may distinguish between hereditary spherocytosis in which the plasma hemoglobin is normal and acquired hemolytic anemia where hemoglobinemia is consistently found. However, the distinction may become blurred by the disappearance of hemoglobinemia as the patient with acquired hemolytic anemia gets well, and by the possibility that hemoglobinemia may occur rarely in the hemolytic crisis of hereditary spherocytosis.[15] In Mediterranean anemia the presence of hemoglobinemia may distinguish between the severe and the mild forms of the disease, but the difference is readily apparent without such a test. The differential diagnosis between sickle-cell anemia and sickle-cell trait is another matter. Here, the clinician may be severely taxed to distinguish the disease from the trait. Our experience is limited, but it seems likely that a demonstration of hemoglobinemia or hemosiderinuria may provide one means of identifying sickle-cell anemia as opposed to sickle-cell trait when confronted with a patient whose blood contains sickle cells.

Considered otherwise than as a point of *differential* diagnosis, the demonstration of hemoglobinemia is unequivocal evidence of abnormal hemolysis and is therefore diagnostic of hemolytic disease.

Prognostic Significance.—In acquired hemolytic anemia the decrease or disappearance of hemoglobinemia appears to be one of the earliest signs of improvement. On several occasions the change occurred without any substantial evidence of clinical improvement. By and large, the variations of the plasma hemoglobin level in a given patient reflected the varying severity of the hemolytic process. Taken alone, the plasma hemoglobin was of little significance in

assessing the severity of the hemolytic disease. In Case 3, with a plasma hemoglobin of 166 mg. per 100 ml., it was readily apparent that blood was being destroyed very rapidly. On the other hand, Case 6 with a plasma hemoglobin level of 62 mg. was less ill than Case 5 with 16 milligrams. This latter patient had the most severe hemolytic disease of any here recorded. As much as a liter of transfused blood per day failed to improve the red cell count. The patient died a few weeks after splenectomy. The spleen showed an extreme degree of erythrophagocytosis. The plasma hemoglobin in this case remained constant throughout the illness at 14 to 16 mg. per 100 milliliters.

Hemoglobinemia in acquired hemolytic anemia is of limited prognostic value. Since it is found during crisis its presence must be regarded as grave, but in such patients other evidences of intense hemolysis are readily apparent.

Pathogenic Significance.—Although hemoglobinemia may be of limited diagnostic and prognostic value, it serves to shed some light upon the hemolytic processes involved in these diseases. It is presumptive evidence that at least a small part of the abnormally hemolysed red cells are destroyed within the circulating blood, although by far the greatest part is probably destroyed in the reticuloendothelial system.

Since hemoglobinemia was not found in hereditary spherocytosis, it is evident that the hemolytic mechanism in this disease differs from that in acquired hemolytic anemia. Apparently, all of the abnormal hemolysis in uncomplicated hereditary spherocytosis occurs in the spleen, since cure of the anemia by splenectomy is the rule.

In acquired hemolytic anemia associated with an abnormal antibody splenectomy is frequently not successful. After splenectomy, the antibody often persists, and so may the hemolytic disease. We are ignorant of the manner in which antibodies bring about the destruction of the red cells, but the process does not necessarily involve the spleen. The presence of hemoglobinemia after splenectomy is a convincing demonstration of this. Intravascular hemolysis is probably a generalized phenomenon which occurs in all parts of the body, not in the spleen alone.

On the other hand, the degree of severity of hemolytic disease persisting in the splenectomized patient with acquired hemolytic anemia may be entirely out of proportion to the relatively minor degree of intravascular blood destruction represented by a plasma hemoglobin concentration of 15 to 62 mg. per 100 ml., as noted in Cases 3 and 6. This indicates that another type of hemolytic process, which is neither splenic nor intravascular, may be involved. This is probably linked up with the activity of the erythrocyte-bound antibody.

The Mechanism of Intravascular Hemolysis.—This probably varies from one form of hemolytic disease to another. The actual physical shock of cardiac systole undoubtedly becomes destructive when the red cells are matted solidly together in the capillaries, as they are in the crisis of sickle-cell anemia or in the presence of a higher titer of cold agglutinin. In Mediterranean anemia, the red cells are easily fragmented.[4] Small "chips" are broken off to form schistocytes. These tiny globules retain hemoglobin within them. It is conceivable that some trace of hemoglobin may be lost at the moment when the fragment

breaks from the parent red cells. Some of these cells, instead of fragmenting, may be destroyed altogether. Increased mechanical fragility of erythrocytes can be demonstrated in many cases of acquired hemolytic anemia associated with an abnormal antibody. In whatever manner the red cell is broken down in these diseases, its hemoglobin is discharged into the surrounding plasma.

The Fate of Plasma Hemoglobin.—The body normally disposes of the hemoglobin of its decomissioned red cells by converting the heme to bilirubin. It is generally accepted that this degradation of hemoglobin occurs in the reticuloendothelial system. The red cell is somehow removed from the circulation, its hemoglobin is destroyed, and the remnants of the heme depart as an iron-free pigment, bilirubinglobin. The iron is then available for storage or re-use, and is transported thence by the iron-binding protein of the plasma. This normal mechanism of hemolysis may be variously referred to as "reticuloendothelial" hemolysis, or "intercellular" or "extravascular" hemolysis. In hemolytic disease the site of hemoglobin conversion depends upon the mechanism of hemolysis. It may be reticuloendothelial, in a sense an intensification of the normal mechanism. It may be splenic, as in the case of hereditary spherocytosis, where some special avidity of the spleen for spherocytes brings about the destruction of these small, round, red cells which could otherwise survive. Thirdly, the site of abnormal hemolysis may be intravascular so that red cells lose their hemoglobin into the circulating plasma. This plasma hemoglobin is not disposed of in the usual manner.

Finch and his co-workers[10] have injected solutions of hemoglobin tagged with radioactive iron into rats and have found that most of this iron has, within a matter of minutes, been deposited in the kidney. On the other hand, tagged iron from the hemoglobin of injured red cells was concentrated in the reticuloendothelial tissues.* It has also been shown that injections of hemoglobin solutions are followed by an elevation of plasma bilirubin.[8] It thus appears that the renal epithelium is capable of degrading hemoglobin to bilirubin. If a relatively small amount of hemoglobin is presented, the kidney is able to contain the iron and dispose of it by the creation of ferritin, the iron-storage protein.[13] From this protein complex the iron is rapidly released to the plasma. However, when a massive amount of hemoglobin is presented to the kidney, either in a paroxysm of hemolysis or by a chronic, continuing intravascular hemolysis, the capacity of the kidney to relieve itself of iron is overtaxed. The iron derived from this hemoglobin is lodged as hemosiderin in the renal epithelium. Some of it is disposed of as hemosiderin in the urine, but much of it is retained as iron-staining pigment in the renal tubular cells. The kidneys in cases of chronic hemoglobinemia are conspicuous by their intense siderosis.[18] They are often larger than normal, suggesting that some embarrassment of function has prompted a compensatory hypertrophy. Renal function tests, however, usually remain unimpaired.[28] The kidney may act as a reticuloendo-

*This work confirms a shrewd surmise of Muir and Dunn[19] made in 1915. As a result of their experiments with hemolytic sera, they insisted upon a distinction between intracellular and extracellular hemolysis. "Phagocytosis and destruction of red corpuscles is the outstanding feature in the spleen. . . . In the kidneys, on the other hand, there is no evidence of phagocytosis, and the iron in the cells of the convoluted tubules is manifestly derived from free hemoglobin in the circulating blood."

thelial organ in the degradation of hemoglobin, but unlike these organs it seems relatively unable to restore to the circulation the iron which it derives from this activity. On the other hand the spleen, as in hereditary spherocytosis, is usually free of hemosiderin.

Destruction of the pigment by the kidney is not the only channel whereby the blood may rid itself of extracorpuscular hemoglobin. A second means is hemoglobinuria which occurs when the plasma hemoglobin concentration exceeds the renal threshold. A third is the formation of methemalbumin.[9] This brown pigment is derived by the combination of plasma albumin with heme groups in which the iron has been oxidized to the ferric state. This pigment is slowly excreted as urobilinogen.

The Relation of Hemosiderinuria to Hemoglobinemia.—Because of the manner in which free hemoglobin is disposed of by the kidney, it would appear inevitable that hemoglobinemia should be accompanied by hemosiderinuria. This in our experience has proved to be the case.

The amounts of hemosiderin in the urine were roughly proportionate to the severity of the hemoglobinemia. Only microscopic amounts were usually found in the urine of patients with plasma hemoglobin values of less than 20 milligrams. When the value was above 40 mg. there was usually a fair-sized button of Prussian blue in the tip of the centrifuge tube. There were exceptions to this, of course. Patients who had a high plasma hemoglobin for long periods of time continued to excrete hemosiderin when the hemoglobinemia was temporarily relieved. This was especially apparent in one patient (Case 14, Table II) with a high titer of cold hemagglutinin. On one occasion, a warm day in winter, she came to the laboratory from her home and was found to have a plasma hemoglobin of 3 mg. per 100 milliliters, but there was a rather considerable deposit of hemosiderin in her urine. On other occasions on cold days she had both hemoglobinemia (15 to 40 mg.) and hemosiderinuria. In children the amount of hemosiderin in the urine was usually less than was found in that of adults with a similar level of plasma hemoglobin. This suggested that the child's kidney was better equipped to restore the iron to the plasma or that, because of the small plasma volume, there was actually less hemoglobin to be cleared from the circulation.

The specificity of hemosiderinuria as an indicator of hemoglobinemia is open to some question. There are reports of hemosiderin in the urine of patients with hemochromatosis,[25, 28] and we have seen it in one patient with this disease; in three others no hemosiderin was found on repeated examinations of the urine. Rous[25] reported the finding of slight and inconstant hemosiderinuria in the urine of one patient with congestive heart failure and of another with terminal nephritis.

Rous also found hemosiderinuria with fair consistency in the urine of patients with pernicious anemia. In all but one of these patients, repeated transfusions had been given. This factor admits some doubt as to the nature of the hemolysis and of the source of the hemosiderin, whether derived from the donor's or the patient's hemoglobin. None of our patients with pernicious anemia had been transfused, and none had hemosiderinuria. On the other hand,

none of our patients was severely anemic. This is probably of some significance. Before the days of liver therapy, indeed, before the use of transfusions, an intense renal siderosis was a well-established pathologic feature of the postmortem studies of pernicious anemia.[16] Such pristine cases of intense severity are rarely encountered nowadays, and few have been studied. Knowledge of the pathogenesis of this anemia has not kept pace with knowledge of its etiology. The hemolytic mechanisms of pernicious anemia are much in need of investigation.

SUMMARY

1. Hemoglobinemia of an intensity not great enough to cause hemoglobinuria has been demonstrated in several hemolytic diseases. It is suggested that the definition of the hemolytic syndrome, as it occurs in acquired hemolytic anemia, sickle-cell anemia, and severe Mediterranean anemia, should be amplified to include a low-grade hemoglobinemia as one of its characteristics. Hemosiderinuria was invariably found when hemoglobinemia was present.

2. Diagnostically the demonstration of hemoglobinemia provides unequivocal evidence of abnormal hemolysis and therefore of hemolytic disease. As a point of differential diagnosis, hemoglobinemia may distinguish between sickle-cell trait and sickle-cell anemia, between mild and severe Mediterranean anemia and, with some reservations, between acquired hemolytic anemia and hereditary spherocytosis.

3. In acquired hemolytic anemia the continued presence of hemoglobinemia must be regarded as an unfavorable prognostic sign for it is usually associated with a severe hemolytic process. However, it was noted that hemoglobinemia was the first sign to diminish or disappear as patients improved.

4. It is pointed out that in many of the hemolytic diseases, multiple mechanisms of abnormal hemolysis may be present.

5. The catabolism of plasma hemoglobin is discussed, together with the significance of hemosiderinuria.

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